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Dividing the empire: boundary chromatin elements delimit the territory of enhancers

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Introduction

Chromatin is the most complex supramolecular organization of the cell. It has a dual role: to compact the chromosomal DNA and to ensure a highly efficient regulation of gene expression. In consequence of the very compact packaging of the DNA, chromatin is highly repressive for transcription. Enhancers are key regulatory elements which can relieve the chromatin-induced repression. The interplay between the chromatin-mediated repression and the activating function of enhancers ensures that the difference between the induced or repressed level of gene expression in eukaryotic cells is several orders of magnitude higher than that in prokaryotic cells. The ability of enhancers to activate a gene independently of their distance from a promoter poses a topological problem: how is the territory of enhancer action delimited? It is supposed that the higher-order chromatin structure divides the genome into topologically constrained loops which are attached to the nuclear matrix. This model implies that the loops are both structural and functional units of the chromatin in the sense that genes under common transcriptional regulation are located within the same loop and regulated by the same set of enhancers (Forrester *et al.*, 1986; Orkin, 1990; Bonifer *et al.*, 1991). In this attractive model, the monogamy of enhancers is ensured by the attachment of the bases of the chromatin loops to the nuclear matrix, which insulates the promoters located within the loop from the regulatory influence of enhancers present in the neighboring loops. Two distinct approaches have been applied to prove the validity of this model. In the structural approach, the main goals were to demonstrate that chromatin is indeed anchored at defined sites to a nuclear matrix, and to identify and characterize DNA sequences at these attachment sites. Structural studies have led to the discovery of scaffold attachment region (SAR) sequences (Mirkovitch *et al.*, 1984). The role of SAR sequences as domain boundary elements, however, is still controversial (Stief *et al.*, 1989; Phi-Van *et al.*, 1990; McKnight *et al.*, 1992; Poljak *et al.*, 1994; Kalos and Fournier, 1995). The functional approach was based on the observation that induction of gene expression is always accompanied by a structural reorganization of the underlying chromatin. A chromatin domain can therefore

be defined as a segment of the genome which exhibits chromatin structural changes upon induction of the gene present in that domain. As the expression of heat shock genes can be induced easily, this approach was first applied to the *Drosophila hsp70* heat shock domain (Udvardy *et al.*, 1985). It was shown that both the proximal and distal sides of the *hsp70* heat shock locus are flanked by novel chromatin structures called 'specialized chromatin structures' (scs and scs'), which are the most remote sequences relative to the *hsp70*-coding sequences that respond with a gross chromatin structural rearrangement after heat induction. It was presumed that scs and scs' structures define the proximal and distal boundaries of the heat shock domain and represent boundary elements with insulating properties. The validity of this approach was justified later by a direct *in vivo* enhancer-blocking assay (Kellum and Schedl, 1992). Since then, a large number of boundary elements have been defined and characterized (Grosveld *et al.*, 1987; Greaves *et al.*, 1989; Bonifer *et al.*, 1990; Holdridge and Dorsett, 1991; Carson and Wiles, 1993; Palmiter *et al.*, 1993; Diaz *et al.*, 1994; Talbot *et al.*, 1994; Jones *et al.*, 1995; May and Enver, 1995; Montoliu *et al.*, 1995; Gdula *et al.*, 1996). On the basis of functional properties, these elements can be divided into two categories: insulators and locus control regions (LCRs).

Insulators

The scs elements and the suppressor of the Hairy-wing [su(Hw)] protein-binding region of the *gypsy* retrotransposon are the best-characterized insulators. The scs and scs' elements are defined by two closely spaced nuclease-hypersensitive (HS) sites arranged around a central nuclease-resistant segment. Characteristic changes occur in the fine structure of this complex HS region in response to heat induction (Udvardy *et al.*, 1985). Additionally, there are localized topoisomerase II sites in the HS complex, and a redistribution of topoisomerase II takes place on heat induction (Udvardy and Schedl, 1993). When DNA fragments carrying either the scs or the scs' region were placed between the *yolk protein-1* (*yp-1*) enhancer and the *hsp70* promoter–*LacZ* fusion gene, the stage-, sex- and tissue-specific activation of the reporter gene by the *yp-1* enhancer was completely blocked. The blocking activity was independent of the orientation of the scs elements. No insulation occurred, however, when the scs elements were inserted upstream of the enhancer (Kellum and Schedl, 1992). Furthermore, the expression of a transgene became copy number-dependent and independent of the site of integration when it was flanked at the 5' end by an scs element and at the 3' end by an scs' element (Kellum and Schedl, 1991). On these criteria, the scs elements are bona fide insulators, because they can fulfill the two most important functions required for a

boundary element: insulation of a gene from the influence of an enhancer and protection of a transgene from chromosomal position effect. Insulators, however, do not have enhancer activity.

Detailed analysis has revealed that DNA fragments associated with the two HS regions of *scs* are essential for full enhancer-blocking activity, while the central AT-rich nuclease-resistant region is dispensable (Vasquez and Schedl, 1994). Deletions which shorten the HS region impaired the insulator activity, but this activity could be fully restored by multimerization of DNA fragments from either HS region. The *scs* insulator is therefore assembled from several independent, functionally redundant DNA sequences, which may be binding sites for sequence-specific DNA-binding proteins. One of these proteins has already been purified, characterized and cloned (Zhao *et al.*, 1995). BEAF-32A recognizes a palindromic sequence which abuts the HS site of the *scs*' boundary element. In an *in vivo* enhancer blocking assay, a 515 bp *scs*' fragment carrying one of these palindromic sequences exhibited strong blocking activity, and even a 7mer repeat of a 48 bp oligonucleotide with a BEAF-binding site produced inhibition. Immunoprecipitation of formaldehyde cross-linked chromatin with a specific antibody developed against the recombinant BEAF-32A protein demonstrated that, *in vivo* also, BEAF-32A binds to *scs*' sequences. Significantly, immunolocalization studies revealed that BEAF-32A is present in several hundred interband regions and at many puff borders on the *Drosophila* polytene chromosome. This indicates that *scs*' is not specific to the hsp70 heat shock domain, but is probably a multipurpose boundary element.

Recently, a second *scs*'-binding protein (BEAF-32B) has been identified and cloned (Hart *et al.*, 1997). It differs from BEAF-32A only in its N-terminus. The unique 5' sequences of these proteins are probably encoded by two independent exons of the same single-copy gene. Three domains have been identified in these proteins. The C-terminal domain is responsible for the observed heterocomplex formation of BEAF-32A and -32B. DNA binding is mediated by the unique N-terminal domains, and this explains the differences observed in DNA-binding specificity detected by DNase I footprinting. The function of the identical middle domain of BEAF-32A and -32B is still not clear.

Another sequence-specific DNA-binding protein (*scs*-binding protein, SBP) which recognizes a 27 bp sequence of *scs* recently has been identified and cloned (M.Gaszner, J.Vasquez and P.Schedl, personal communication). Multimers of the 27 bp recognition sequence exhibited *in vivo* enhancer-blocking activity. Mutations in this oligonucleotide disrupted the *in vitro* binding of the recombinant SBP and eliminated the enhancer-blocking activity *in vivo*. A chromatin immunoprecipitation assay revealed that SBP binds *in vivo* to a region of *scs* which corresponds to the *in vitro* binding site. The vital role of the boundary function is supported by the observation that the strong loss-of-function mutations of the SBP gene are recessive cell autonomous lethal, while weaker alleles have pleiotropic effects on development. This is consistent with the assumption that the *scs*, like *scs*', is a multipurpose insulator involved in the regulation of several different

genes, and SBP has an important role in the maintenance of chromatin architecture.

Insertion of the *gypsy* retrotransposon causes a variety of mutations in *Drosophila*. Some of these mutations exhibit typical tissue or developmental specificity. Analysis of mutations induced by insertion of the *gypsy* into different regions of the *yellow* gene revealed that the phenotype of the mutation depended on the location of the integration site relative to the position of tissue-specific *yellow* enhancers. Enhancers located upstream of the integration site relative to the position of the promoter were inactivated. A short region of the *gypsy* transposon carrying a repeated octamer sequence motif flanked by AT-rich sequences is responsible for the mutations (Geyer *et al.*, 1988; Smith and Corces, 1995). Insertion of this short sequence between an enhancer and the promoter blocks the activity of the enhancer (Holdridge and Dorsett, 1991; Jack *et al.*, 1991; Geyer and Corces, 1992). A transgene flanked on both sides by this short sequence is protected from the chromosomal position effect (Roseman *et al.*, 1993). On these criteria, the *gypsy* is a true insulator. The repeated octamer sequence motif is the binding site of Su(Hw), a *Drosophila* nuclear protein with 12 zinc fingers in the central domain, with a leucine zipper domain and with two highly acidic domains at the N- and C-terminal segments of the protein (Spana *et al.*, 1988; Mazo *et al.*, 1989; Dorsett, 1990). Mutation analysis revealed that the zinc finger and the leucine zipper motifs are critical for enhancer blocking (Harrison *et al.*, 1993).

The modifier of *mdg4* [mod(*mdg4*)] protein interacts with Su(Hw), this interaction being essential for the insulator activity (Gerasimova *et al.*, 1995). The phenotype of *gypsy*-induced mutations at a variety of loci is modified in a mod(*mdg4*) background (Georgiev and Gerasimova, 1989). Analysis of the effect of the mod(*mdg4*) mutation on the *gypsy*-induced *y*² phenotype of the *yellow* gene led to the postulation that the binding of the Su(Hw)-mod(*mdg4*) protein complex to the *gypsy* insulator ensures a unidirectional enhancer-blocking activity, whereas Su(Hw) alone converts the unidirectional insulator to a bi-directional silencer which inactivates all the tissue-specific enhancers of the *yellow* gene, irrespective of the site of insertion of the *gypsy* retrotransposon (Gerasimova *et al.*, 1995). This change, however, is not universal; there are genes where the mod(*mdg4*) mutation increases the *gypsy*-induced phenotype (Georgiev and Kozycina, 1996). This suggests that mod(*mdg4*) interacts both with Su(Hw) and with other chromosomal proteins, and these latter interactions are gene specific.

Several different models have been proposed with regard to the mode of action of insulators (Figure 1). The domain boundary model attempted to link the physical organization of chromosomes with the most important functional properties of insulators. In this model (Figure 1A), the three-dimensional folding of a chromatin domain, i.e. a DNA segment delimited by insulator elements, would facilitate productive interactions between regulatory elements located within the domain, while it would preclude equivalent interactions between these elements and regulatory elements outside the domain (Udvardy *et al.*, 1985). In the 'tracking' model (Figure 1B), transcription factors bound to enhancer elements track along the DNA in search of target promoters. Insulator-bound sequence-

Fig. 1. Models of insulation. Different models of insulation, as described in detail in the text, are shown. E, enhancer; Pr, promoter; Ins, insulator.

specific DNA-binding proteins block transcription factors from reaching the target promoter (Chung *et al.*, 1993). Insulators may also reduce the accessibility of promoters to complexes formed on enhancer elements (Figure 1C; Chung *et al.*, 1993). In the transcriptional decoy model (Figure 1D), the complex formed on the insulator may function as an enhancer sink by attracting the complex assembled on the enhancer into a non-productive interaction (Geyer, 1997). The data available today are insufficient to allow an unambiguous distinction between these models. One important new observation should be borne in mind in connection with all the models proposed for the mode of insulator action: an enhancer prevented from acting on one promoter by the insertion of an insulator remains fully active for another uninsulated promoter (Figure 1E; Cai and Levine, 1995; Scott and Geyer, 1995). This indicates that insulators do not propagate the formation of a repressive chromatin structure.

Recent studies suggest that proteins involved in chromatin domain border formation may also be involved in the overall organization of the nucleus. The punctated immunostaining pattern of mod(mdg4) and su(Hw) proteins in the nucleus of *Drosophila* imaginal disc cells and their co-localization with lamin suggest that many

individual binding sites for these proteins come together in the same subnuclear region, and protein components of the insulators might establish this organization by attaching the chromatin fiber to the nuclear matrix (Gerasimova and Corces, 1998).

There is some debate as regards the involvement of chromatin structure in the process of insulation. The complex HS structure observed on scs and scs' sequences suggests that the blocking activity of insulators requires a supporting chromatin architecture. It has been shown recently, however, that scs or scs' elements can block enhancer-activated transcription on a plasmid DNA microinjected into *Xenopus laevis* oocytes (Dunaway *et al.*, 1997; Krebs and Dunaway, 1998). Although the enhancer-blocking activity appeared earlier during the microinjection experiment than the completion of the nucleosome assembly, this is not a direct indication that enhancer blocking does not require a chromosomal context. The microinjection-based assay cannot measure separately the transcriptional activity of a fully assembled, partially assembled or unassembled plasmid DNA. Enhancer activity is known to be almost 50-fold higher on chromatin-assembled plasmid DNA as compared with naked DNA (Workman *et al.*, 1991). If a similar dependence of the insulator activity on the structure of template DNA is assumed, a small fraction of nucleosome-assembled plasmid may confuse the results obtained in the microinjection-based assay.

Locus control regions

The discovery of LCRs was a joint success of researches in the fields of molecular biology and molecular medicine. The initial failure of transgenic experiments to achieve high-level, copy number-dependent transgene expression suggested that, besides the enhancers identified in transient expression experiments as being essential for the appropriate regulation of the transgene, additional, hitherto unidentified regulatory sequences are also required. The identification of these missing sequences was based on the observation that the deletion of DNA sequences far upstream from the β -globin gene in Hispanic ($\gamma\delta\beta$) thalassemia resulted in a severely reduced β -globin expression. Analysis of the chromatin architecture of the DNA segment deleted in ($\gamma\delta\beta$) thalassemia revealed the presence of five tissue-specific DNase HS structures (Groudine *et al.*, 1983; Tuan *et al.*, 1985). Transgenes of the β -globin gene flanked by the DNA segment carrying these HS sites resulted in the expected high-level, copy number-dependent expression, which was independent of the site of integration (Grosveld *et al.*, 1987). The 15 kb DNA segment with these HS sites was called the LCR. Several different LCRs subsequently have been identified and characterized.

The structures and functions of the individual components of the LCRs have been studied by different techniques. Sequence analysis demonstrated that there are multiple erythroid-specific and ubiquitous transcription factor-binding motifs in the HS sites (Hardison *et al.*, 1997). It is important to note that, although in different numbers and spatial arrangements, each of the HS sites contains the same set of transcription factor-binding sites. Some of these binding sites are also present in the enhancers

responsible for the tissue-specific and developmental stage-specific expression of the different globin genes. Transient or stable expression studies and transgenic experiments revealed that HS site 2 of the human globin LCR exerts enhancer activity (Tuan *et al.*, 1987; Collis *et al.*, 1990). HS site 3 will cause high-level expression of the β -globin gene after stable integration, but will not enhance its transient expression (Collis *et al.*, 1990), while HS sites 1 and 4 have no enhancer activity (Pruzina *et al.*, 1991). Deletion of the individual HS sites only mildly reduces the expression of the globin genes, indicating that the HS sites cooperatively regulate the expression of all the genes of the locus. The coordinated enhancer activity of these HS sites is believed to be responsible for the chromatin domain opening activity, which is the most characteristic property of the LCRs. HS site 5 of the human globin LCR (Li and Stramatoyannopoulos, 1994), HS site 4 of the chicken globin LCR (Chun *et al.*, 1993; Pikaart *et al.*, 1998) and element A of the chicken lysozyme LCR (Bonifer *et al.*, 1994) exhibit *in vivo* enhancer-blocking activity characteristic of insulators. The enhancer-blocking activity of the chicken HS site 4 is retained in *Drosophila*. These HS sites with insulator activity may have a pivotal role in the position-independent expression of the transgenes, while the domain opening activity may ensure the high-level and copy number-dependent expression.

Despite the accumulation of a large body of information on the structures of LCRs, and on their interactions with sequence-specific DNA-binding proteins, the detailed molecular mechanism(s) of the mode of their protective action is far from being resolved. Two different models have been proposed (Martin *et al.*, 1996; Dillon *et al.*, 1997). Although there is a sharp difference in the interpretation of the data which form the basis of these models, the contradictions, as shown below, are not irreconcilable.

The competition model is based on a detailed analysis of the *in vivo* dynamics of transcription complex interactions which occur in the developmentally regulated chromatin architecture of the globin locus. All five human β -globin genes are under the regulatory control of the globin LCR. These genes are arranged in the 70 kb globin locus in the sequence of their developmental expression. It was presumed earlier that the different HS elements of the LCR interact with different genes of the locus, and each HS region is responsible separately for the developmental regulation of a given globin gene (Engel, 1993). However, it is now generally accepted that all the HS elements of the LCR act together, probably by forming a holocomplex. This followed from the observation that each of the genes required all of the HS elements for full activity (Bungert *et al.*, 1995; Fiering *et al.*, 1995; Hug *et al.*, 1996). *In situ* hybridization with gene-specific hybridization probes on individual embryonic and fetal transgenic erythroid cells can identify those globin genes which are expressed at a given time, at individual alleles, in individual cells. The *in situ* hybridization experiments detected single gene signals in the majority of the cells. Kinetic analysis based on the rate of primary transcript processing revealed that multigene signals present in a small fraction of cells represented a recent switch of transcription of different globin genes and not concurrent transcription from multiple genes. From the aspect of the chromatin interaction

dynamics these data suggested that although the LCR holocomplex interacts with only a single promoter at a time, it can switch back and forth between the different promoters of the globin locus by a flip-flop mechanism, and this switching occurs continuously throughout all the developmental stages of globin gene expression (Wijgerde *et al.*, 1995). The prevailing LCR-promoter interaction in a given period of development is determined by the abundance of gene-specific transcription factor(s) and the distance of the promoter from the LCR (Dillon *et al.*, 1997). The transition from a given developmental phase to the subsequent one is governed by the change of transcription factor abundance, which is accompanied by a gradual transition in the prevailing LCR-promoter interaction. However, this transition does not abolish the potential for occurrence of the LCR-gene interaction which was predominant in the previous developmental phase. It has been calculated that *in vivo* the LCR-gene interaction is switched every 10–15 min, and this flip-flop does not require the replication of DNA. The continuous availability of all the genes of the globin locus for a single, coordinately acting LCR requires continuous access for the governing LCR toward all the regulatory entities (promoters and enhancers) of all the genes of the globin locus. This means that the globin chromatin domain in erythroid cells should be multihooked, i.e. all the regulatory entities of the 70 kb globin locus should maintain dynamic interactions with the LCR (Gribnau *et al.*, 1998), and the intervening DNA sequences should be looped out.

How is this complex chromatin architecture formed? The main problem in the looping model of enhancer action is that it does not take into account the energetic burden of DNA looping. The concentration of DNA in the nucleus of a human cell is ~7–8 mg/ml. The viscosity of a megabase pair long chromatin fiber in this concentration range is very high. What will provide the energy required to move a segment of the chromatin several thousands of base pairs away? Transcription factors, having neither ATPase nor GTPase domains, cannot provide the energy. It is much more reasonable to suppose that the complex chromatin architecture of the whole globin locus is formed coordinately at the time when the commitment for erythroid-specific differentiation occurs. This coordinated formation of the chromatin architecture is probably initiated by the appearance of high-affinity, sequence-specific DNA-binding protein(s) which specifically recognize the globin LCR and tether the DNA at the LCR to a protein frame (we may call this the 'nucleoskeleton' to distinguish it from the nuclear matrix). This process probably requires the disruption of the nucleosomal organization of the chromatin at the site of tethering, and it may therefore occur during the replication of DNA. Alternatively, the appearance of special chromatin remodeling factor(s) may catalyze this rearrangement. Tethering the DNA at the LCR by this high-affinity DNA-binding protein(s) may initiate the looping of the globin locus (Figure 2). If the same or different sequence-specific DNA-binding protein(s) recognize and tether the DNA to the nucleoskeleton at the different intradomain enhancers of the globin locus, the multihooked chromatin architecture can be formed coordinately. GATA- and CACC-binding motifs are present both in the enhancers flanking the globin genes and also in the HS sites of the LCR. The higher

Fig. 2. Model for the establishment of a whole-domain, multihooked chromatin architecture. During the replication of DNA, occurring as DNA is reeled through a nucleoskeleton-bound 'replication factory' (Cook, 1991), DNA is tethered to the nucleoskeleton at the LCR and at internal enhancer sequences due to the binding of sequence-specific DNA-binding proteins. The dominant role of LCR in the replication of the globin locus is well documented (Forrester *et al.*, 1990; Aladjem *et al.*, 1995). R.F., replication factory; E₁ and E₂, internal enhancers of the locus.

concentration of these factor-binding sites in the LCR may be responsible for their predominant role in the formation of the primary contacts at the LCR, while the presence of these sites in the enhancers present in the vicinity of the globin genes may ensure the coordinated formation of the whole-domain, multihooked chromatin architecture which establishes the structural basis of all the dynamic contacts between the LCR and the different globin genes. This newly formed multihooked chromatin architecture should be stabilized subsequently by non-histone chromosomal proteins and heritably maintained until the high-affinity binding protein(s) is present in the cell. This multihooked chromatin organization is a dynamic structure, its formation is dependent on the transcriptional activation of the domain, and it should be clearly distinguished from the chromatin loops organized by the SAR sequences.

The role of cellular differentiation in the reorganization of the chromatin architecture and in the transcriptional activation of a domain is well documented in the case of the chicken lysozyme locus (Bonifer *et al.*, 1997).

When a transgene carrying an intact LCR integrates close to the centromeric heterochromatin, the LCR completely protects the locus from the repressive effect of heterochromatinization. Pericentromeric integration of a transgene carrying the globin locus with an incomplete LCR results in a severely reduced globin gene expression, this frequently being due to clonally inherited position

effect variegation (Milot *et al.*, 1996). This suggests that there is strong competition for the establishment of heritably stabilized higher-order chromatin structures. Heterochromatinization, which is a highly penetrative process, is totally blocked in the presence of a complete LCR, probably because of the very high stability of the presumed complex formed on the LCR. With an incomplete LCR, the formation of this primary complex is slower, and/or the complex is less stable. As it is a stochastic process, the timing of the formation of this primary complex will vary from cell to cell. In those cells where the binding of the presumed LCR-binding protein(s) to its cognate recognition sequence precedes the spread of heterochromatinization, epigenetic mechanisms will stabilize this LCR-specified primary interaction, resulting in the formation of a clonally inherited stable higher order chromatin structure which is permissive for globin expression. In those cells where the spread of heterochromatinization precedes the primary LCR-protein interaction, however, heterochromatin will be stabilized and heritably transmitted, resulting in the appearance of position effect variegation.

Similar results were obtained after the deletion of HS site 3 of the human *CD2* gene LCR (Festenstein *et al.*, 1996). Transgenes with a deleted HS site 3 exhibited variegated expression when integrated in the centromere. DNase I HS site analysis of the nuclei of fluorescence-activated cell sorting (FACS)-separated CD2⁺ and CD2⁻ cells of the variegating cell population revealed that HS site 1 was completely missing in CD2⁻ cells, whereas it was fully retained in CD2⁺ cells. This is a direct indication of the epigenetic stabilization of a clonally inherited stable higher order chromatin structure.

Deletion of essential DNA sequences from the LCR is not the only way to influence the competition between the formation of different higher-order chromatin structures. Similar results can be achieved by eliminating a protein component essential for the function of a regulatory chromatin element. Thus, deletion of mod(mdg4) results in a variegated expression pattern of gypsy-induced mutations similar to those chromosomal rearrangements that bring the gene juxtaposed to heterochromatic sequences (Gerasimova *et al.*, 1995).

The second model proposed for the mechanism of LCR action was based on the 'probability' theory of enhancer activation. When a γ -globin promoter-*LacZ-neo*^R fusion gene with or without a flanking enhancer sequence was stably integrated into K562 erythroleukemia cells, a flanking 5'-HS site 2 from the β -globin LCR, which is a strong erythroid-specific enhancer, produced a 23-fold increase of G418-resistant colonies (Walters *et al.*, 1995). When FACS-separated β -gal-positive cells were assayed by a quantitative β -gal activity test, no differences were observed between the enhancer-containing and enhancerless cells. Thus, an enhancer, like a binary switch, increases the probability of expression of an integrated construct, but it does not substantially increase the level of expression. When the enhancer was removed *in vivo* by flp recombinase from stably integrated β -gal-expressing cells carrying the γ -globin promoter-*LacZ-neo*^R-enhancer construct, there was only a slight decline in expression level, but there was a dramatic increase in the rate at which the β -gal expression was silenced when cells were

maintained under non-selective conditions (Walters *et al.*, 1996). This means that enhancers have a dual role during the stable integration of DNA: to increase the probability of the establishment of a permissive chromatin environment and to protect the integrated DNA from the repressive effect of the surrounding chromatin. A similar mechanism has been proposed for the mode of action of LCRs. According to this model (Martin *et al.*, 1996), the LCR, as a highly specialized enhancer, increases the establishment and maintenance of a transcriptionally permissive chromatin environment. This model is supported by the observation that the transgenic expression of β -galactosidase under the control of globin promoters linked to α -globin HS-40 enhancer (which is similar to the β -globin LCR) is heterocellular in all mouse lines, the fact that the number of expressing cells varies greatly between different transgenic lines and that the percentage of expressing cells is constant within a transgenic line (Robertson *et al.*, 1995). No looping is required in this model; the arrangement of the genes within the locus, and not their proximity to the LCR or the function of the LCR, is responsible for the developmental switching: the most 5' gene is transcribed first and suppresses a downstream gene. Switching is achieved by the formation of a different stable chromatin structure, this process being clonally inherited and independent of the transcription factor environment. This model, however, does not explain how a different stable chromatin structure is formed, what the mechanism of this transition is or what kinds of DNA elements and *cis*-acting proteins are involved. Neither does the model explain the mechanism of the fine interplay between the LCR and gene-proximal regulatory elements (Stamatoyannopoulos *et al.*, 1997), nor can it interpret the competition observed between identical globin genes inserted at different locations within the globin locus (Dillon *et al.*, 1997). The heterocellular transgene expression mentioned above may simply be due to the incomplete LCR function of α -globin HS-40 enhancer (Gourdon *et al.*, 1994), similarly to the position effect variegation of transgenes, which is always a consequence of LCR impairment.

How can an enhancer increase the number of transcriptionally active stable integrants? During the stable integration of a reporter construct, there is a chance for the formation of a higher-order chromatin structure which is permissive for the expression of the integrated reporter gene. If the transfected cell line contains a set of transcription factors which can interact both with the LCR present in the vicinity of the integration site and with the promoter and/or enhancer of the reporter gene, dynamic contact between the HS sites, and accompanying looping out of the intervening DNA may occur. LCRs can activate heterologous reporter gene–promoter–enhancer constructs. Addition of the human *CD2* LCR to a β -globin fragment which carries its promoter and enhancers will induce the expression of β -globin in the T cells of the transgenic animal (Greaves *et al.*, 1989). Similarly, T-cell receptor α LCR activates the above β -globin fragment in transgenic animals. A complete T-cell receptor α LCR with eight HS sites results in tissue-specific expression of the transgene, but removal of a segment of the LCR carrying three HS sites abolishes the tissue specificity and results in a ubiquitous expression in all tissues (Ortiz *et al.*, 1997). It

is presumed that the T-cell receptor α LCR is a bifunctional element, regulating both the T-cell receptor α gene and the adjacent *Dad1* antiapoptosis gene which is ubiquitously expressed at a high level (Hong *et al.*, 1997). Thus the assumption that the integrated gene will interact with the LCR present in the vicinity of the integration site is plausible. Although it is not evident that the regulation of all loci requires LCR activity, the widespread occurrence of position effect of the integrated transgenes strongly suggests it. As the formation of protein–protein interactions between the HS sites is probably the rate-limiting step, the stochastic occurrence of this event is greatly facilitated if the integrated DNA contains an enhancer, because of the presence of a large number of high-affinity protein-binding sites. This assumption explains both the ‘probability’ action of enhancers and the influence of the chromatin environment at the site of integration. In consequence of the lack of certain common transcription factor-binding sites, the interaction between an enhancer and an unrelated LCR (i.e. one present at the site of integration) can rarely be as strong as that between cognate regulatory elements. This may explain why the level of transgene expression integrated without its cognate LCR frequently is very low.

Once the contacts between the HS sites are formed, epigenetic mechanisms should stabilize the higher-order chromatin structure. This is probably a second, independent, but equally important step in the formation of the transcriptionally competent chromatin architecture. The independent role of this stabilization step is supported by the observation that *in vivo* removal of the enhancer by flip recombinase from the stably integrated γ -globin promoter–*LacZ*–*neo*^R–enhancer construct does not arrest the expression immediately, but results in a gradual silencing of the gene (Walters *et al.*, 1996). The silencing is almost always irreversible. This again supports the assumption that the assembly of a transcriptionally active, multihooked chromatin structure is a rare event, which occurs only under special circumstances (major developmental transitions, integration of DNA, etc.).

A similar ‘all-or-none’ model of HS site formation was observed on the chicken erythroid-specific β^A/ϵ globin gene enhancer. The probability of HS site formation after stable integration into a chicken erythroid cell line of a β -globin fragment carrying different enhancer mutations was proportional to the number of unmutated erythroid factor-binding sites (Boyes and Felsenfeld, 1996).

The question as to whether there is a conceptual difference between the function of insulators or the LCR remains. The observation that the LCR of the human and chicken globin domains includes an HS site with typical insulator property suggests that the LCR is a more complex, more specialized type of insulator. While the scs or the *gypsy* insulators can function in a large variety of chromatin domains, where insulation from the repressive effect of the surrounding chromatin is sufficient for the proper gene expression, LCR, besides insulating the locus from the position effect, performs the much more complex task of coordinating the tissue- and developmental stage-specific expression program of the domain.

Future prospects

Endeavors to understand all the details of the boundary function are greatly hampered by the technical difficulties

of studying these entities. *In vitro* test systems are not suitable, and the currently available *in vivo* tests are slow and laborious. Although our knowledge of the mode of action of insulators is less advanced than our understanding of the molecular details of the LCR function, genetic approaches that exploit the high power of *Drosophila* genetics will essentially speed up the molecular dissection of insulators (Gyurkovics *et al.*, 1990). The generation of second-site mutations which enhance or suppress the insulator function is an ideal system for identification of all the interacting proteins involved in insulator function. *In vivo* studies on boundary functions have been done predominantly in flies (insulators) or mice (LCR). The establishment of *in vivo* test systems in other species will certainly improve our chances of obtaining a more complete understanding of how these chromatin elements regulate the gene expression. For elucidation of the boundary function, significant progress is required regarding the understanding of the molecular details of the 'repressive effect of the surrounding chromatin'. The difference between euchromatic and heterochromatic silencing is still not known. It is vital to find a direct test system to prove or disprove the looping of DNA within chromatin. Perhaps the fusion of a domain to an enhancer-binding protein which can 'label' the DNA can reveal the interaction of the fusion protein with a remote DNA sequence. Our limited knowledge of the higher-order chromatin structure makes an understanding of the boundary function a huge challenge. Spectacular advances have occurred in this field during recent years, but further combined efforts of molecular biology and genetics will be required to achieve this goal.

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References

- Aladjem, M.I., Groudine, M., Brody, L.L., Dieken, E.S., Fournier, R.E.K., Wahl, G.M. and Epner, E.M. (1995) Participation of the β -globin locus control region in initiation of DNA replication. *Science*, **270**, 815–819.
- Bonifer, C., Vidal, M., Grosveld, F. and Sippel, A.E. (1990) Tissue specific and position independent expression of the complete gene domain for chicken lysozyme in transgenic mice. *EMBO J.*, **9**, 2843–2848.
- Bonifer, C., Hecht, A., Saueressig, H., Winter, D. and Sippel, A.E. (1991) Dynamic chromatin: the regulatory domain organization of eukaryotic gene loci. *J. Cell Biochem.*, **47**, 99–108.
- Bonifer, C., Yannoutsos, N., Kruger, G., Grosveld, F. and Sippel, A.E. (1994) Dissection of the locus control function located on the chicken lysozyme gene domain in transgenic mice. *Nucleic Acids Res.*, **22**, 4202–4210.
- Bonifer, C., Jagle, U. and Huber, M.C. (1997) The chicken lysozyme locus as a paradigm for the complex developmental regulation of eukaryotic gene loci. *J. Biol. Chem.*, **272**, 26075–26078.
- Boyes, J. and Felsenfeld, G. (1996) Tissue-specific factors additively increase the probability of the all-or-none formation of a hypersensitive site. *EMBO J.*, **15**, 2496–2507.
- Bungert, J., Dave, U., Lieuw, K.H., Shavit, J.A., Liu, Q. and Engel, J.D. (1995) Synergistic regulation of human β -globin gene switching by locus control elements HS3 and HS4. *Genes Dev.*, **9**, 3083–3096.
- Cai, H. and Levine, M. (1995) Modulation of enhancer–promoter interactions by insulators in the *Drosophila* embryo. *Nature*, **376**, 533–536.
- Carson, S. and Wiles, M. (1993) Far upstream regions of class II MHC Ea are necessary for position independent copy number dependent expression of Ea transgenesis. *Nucleic Acids Res.*, **21**, 2065–2072.
- Chung, J.H., Whiteley, M. and Felsenfeld, G. (1993) A 5' element of the chicken β -globin domain serves as an insulator in human erythroid cells and protects against position effects in *Drosophila*. *Cell*, **74**, 505–514.
- Collis, P., Antoniu, M. and Grosveld, F. (1990) Definition of the minimal requirements within the human β -globin gene and the dominant control region for high level expression. *EMBO J.*, **9**, 233–240.
- Cook, P.R. (1991) The nucleoskeleton and the topology of replication. *Cell*, **66**, 627–635.
- Diaz, P., Cado, P. and Winoto, A. (1994) A locus control region in the T cell receptor α/δ locus. *Immunity*, **1**, 207–217.
- Dillon, N., Trimborn, T., Strouboulis, J., Fraser, P. and Grosveld, F. (1997) The effect of distance on long-range chromatin interactions. *Mol. Cell*, **1**, 131–139.
- Dorsett, D. (1990) Potentiation of polyadenylation site by a downstream protein–DNA interaction. *Proc. Natl Acad. Sci. USA*, **87**, 4373–4377.
- Dunaway, M., Hwang, J.Y., Xiong, M. and Yuen, H.-L. (1997) The activity of the scs and scs' insulator elements is not dependent on chromosomal context. *Mol. Cell. Biol.*, **17**, 182–189.
- Engel, J.D. (1993) Developmental regulation of human β -globin gene transcription: a switch of loyalties? *Trends Genet.*, **9**, 304–309.
- Festenstein, R., Tolaini, M., Corbella, P., Mamalaki, C., Parrington, J., Fox, M., Miliou, A., Jones, M. and Kioussis, D. (1996) Locus control region function and heterochromatin-induced position effect variegation. *Science*, **271**, 1123–1125.
- Fiering, S., Epner, E., Robinson, K., Zhuang, Y., Telling, A., Hu, M., Martin, D.I.K., Enver, T., Ley, T. and Groudine, M. (1995) Targeted deletion of 5'HS2 of the murine β -globin locus reveals that it is not essential for proper regulation of the β -globin locus. *Genes Dev.*, **9**, 2203–2213.
- Forrester, W.C., Thompson, C., Elder, T. and Groudine, M. (1986) A developmentally stable chromatin structure in the human β -globin cluster. *Proc. Natl Acad. Sci. USA*, **83**, 1359–1363.
- Forrester, W.C., Epner, E., Driscoll, M.C., Enver, T., Brice, M., Papayannopoulou, T. and Groudine, M. (1990) A deletion of the β -globin locus activation region causes a major alteration in chromatin structure and replication across the entire β -globin locus. *Genes Dev.*, **4**, 1637–1649.
- Gdula, D.A., Gerasimova, T.I. and Corces, V.G. (1996) Genetic and molecular analysis of the gypsy chromatin insulator of *Drosophila*. *Proc. Natl Acad. Sci. USA*, **93**, 9378–9383.
- Georgiev, P.G. and Gerasimova, T.I. (1989) Novel genes influencing the expression of the yellow locus and *mdg4* (gypsy) in *Drosophila melanogaster*. *Mol. Gen. Genet.*, **220**, 121–126.
- Georgiev, P.G. and Kozycina, M. (1996) Interaction between mutations in the suppressor of *Hairy-wing* and *modifier of mdg4* genes of *Drosophila melanogaster* affecting the phenotype of gypsy-induced mutations. *Genetics*, **142**, 425–436.
- Gerasimova, T.I. and Corces, V.G. (1998) Polycomb and trithorax group proteins mediate the function of a chromatin insulator. *Cell*, **92**, 511–521.
- Gerasimova, T.I., Gdula, D.A., Gerasimov, D.V., Simonova, O. and Corces, V.G. (1995) A *Drosophila* protein that imparts directionality on a chromatin insulator is an enhancer of position-effect variegation. *Cell*, **82**, 587–597.
- Geyer, P.K. (1997) The role of insulator elements in defining domains of gene expression. *Curr. Opin. Genet. Dev.*, **7**, 242–248.
- Geyer, P.K. and Corces, V.G. (1992) DNA position-specific repression of transcription by a *Drosophila* Zn finger protein. *Genes Dev.*, **6**, 1865–1873.
- Geyer, P.K., Green, M.M. and Corces, V.G. (1988) Mutant gene phenotypes mediated by a *Drosophila melanogaster* retrotransposon require sequences homologous to mammalian enhancers. *Proc. Natl Acad. Sci. USA*, **85**, 8593–8597.
- Gourdon, G., Sharpe, J.A., Wells, D., Wood, W.G. and Higgs, D.R. (1994) Analysis of a 70 kb segment of DNA containing the human ζ and α -globin genes linked to their regulatory element (HS-40) in transgenic mice. *Nucleic Acids Res.*, **22**, 4139–4147.
- Greaves, D.R., Wilson, F.D., Lang, G. and Kioussis, D. (1989) Human CD2 3' flanking sequences confer high level, T-cell specific, position-independent gene expression in transgenic mice. *Cell*, **56**, 979–986.
- Gribnau, J., de Boer, E., Trimborn, T., Wijgerde, M., Milot, E., Grosveld, F. and Fraser, P. (1998) Chromatin interaction mechanism of transcriptional control *in vivo*. *EMBO J.*, **17**, 6020–6027.
- Grosveld, F., van Assendelft, G.B., Graeves, D.R. and Kollias, G. (1987) Position independent, high-level expression of the human β -globin gene in transgenic mice. *Cell*, **51**, 975–985.

- Groudine, M., Kohwi-Shigematsu, T., Gelinas, R., Stramatoyannopoulos, G. and Papayannopoulou, T. (1983) Human fetal to adult hemoglobin switching: changes in chromatin structure of the β -globin locus. *Proc. Natl Acad. Sci. USA*, **80**, 7551–7555.
- Gyurkovics, H., Gausz, J., Kummer, J. and Karch, F. (1990) A new homeotic mutation in the *Drosophila* bithorax complex removes a boundary separating two domains of regulation. *EMBO J.*, **9**, 2579–2585.
- Hardison, R., Slightom, J.L., Gumucio, D.L., Goodman, M., Stojanovic, N. and Miller, W. (1997) Locus control regions of mammalian β -globin gene clusters: combining phylogenetic analyses and experimental results to gain functional insights. *Gene*, **205**, 73–94.
- Harrison, D.A., Gdula, D.A., Coyne, R.S. and Corces, V.G. (1993) A leucine zipper domain of the suppressor of Hairy-wing protein mediates its repressive effect on enhancer function. *Genes Dev.*, **7**, 1966–1978.
- Hart, C.M., Zhao, K. and Laemmli, U.K. (1997) The scs' boundary element: characterization of boundary element-associated factors. *Mol. Cell. Biol.*, **17**, 999–1009.
- Holdridge, C. and Dorsett, D. (1991) Repression of *hsp70* heat shock gene transcription by the suppressor of Hairy-wing protein of *Drosophila melanogaster*. *Mol. Cell. Biol.*, **11**, 1894–1900.
- Hong, N.A., Cado, D., Mitchell, J., Ortiz, B.D., Hsieh, S.N. and Winoto, A. (1997) A targeted mutation at the T-cell receptor α/δ locus impairs T-cell development and reveals the presence of the nearby antiapoptosis gene *Dad1*. *Mol. Cell. Biol.*, **17**, 2151–2157.
- Hug, B., Wesselschmidt, R.L., Fiering, S., Bender, M.A., Groudine, M. and Ley, T.J. (1996) Analysis of mice containing a targeted deletion of the β -globin locus control region 5'HS-3. *Mol. Cell. Biol.*, **16**, 2906–2912.
- Jack, J., Dorsett, D., DeLotto, Y. and Liu, S. (1991) Expression of the cut locus in the *Drosophila* wing margin is required for cell type specification and is regulated by a distal enhancer. *Development*, **113**, 735–748.
- Jones, B.K., Monks, B.R., Liebhauer, S.A. and Cooke, N.E. (1995) The human growth hormone gene is regulated by a multicomponent locus control region. *Mol. Cell. Biol.*, **15**, 7010–7021.
- Kalos, M. and Fournier, R.E.K. (1995) Position independent transgene expression mediated by boundary elements from the apolipoprotein B chromatin domain. *Mol. Cell. Biol.*, **15**, 198–207.
- Kellum, R. and Schedl, P. (1991) A position-effect assay for boundaries of higher order chromosomal domains. *Cell*, **64**, 941–950.
- Kellum, R. and Schedl, P. (1992) A group of scs elements function as domain boundaries in an enhancer-blocking assay. *Mol. Cell. Biol.*, **12**, 2424–2431.
- Krebs, J.E. and Dunaway, M. (1998) The scs and scs' insulator elements impart a *cis* requirement on enhancer–promoter interactions. *Mol. Cell*, **1**, 301–308.
- Li, Q. and Stramatoyannopoulos, G. (1994) Hypersensitive site 5 of the human β locus control region functions as a chromatin insulator. *Blood*, **84**, 1399–1401.
- Martin, D.I.K., Fiering, S. and Groudine, M. (1996) Regulation of β -globin gene expression: straightening out the locus. *Curr. Opin. Genet. Dev.*, **6**, 488–495.
- Mazo, A.M., Mizrokhi, L.J., Karavanov, A.A., Sedkov, Y.A., Krichevskaja, A.A. and Ilyin, Y.V. (1989) Suppression in *Drosophila*: *su(Hw)* and *su(f)* gene products interact with a region of *gypsy* (*mdg4*) regulating its transcriptional activity. *EMBO J.*, **8**, 903–911.
- May, G. and Enver, T. (1995) Targeting gene expression to haemopoietic stem cells: a chromatin-dependent upstream element mediates cell type-specific expression of the stem cell antigen CD34. *EMBO J.*, **14**, 564–574.
- McKnight, R.A., Shamay, A., Sankaran, L., Wall, R.J. and Henninghausen, L. (1992) Matrix-attachment regions can impart position independent regulation of a tissue-specific gene in transgenic mice. *Proc. Natl Acad. Sci. USA*, **89**, 6943–6947.
- Milot, E. et al. (1996) Heterochromatin effects on the frequency and duration of LCR-mediated gene transcription. *Cell*, **87**, 105–114.
- Mirkovitch, J., Mirault, M.-E. and Laemmli, U.K. (1984) Organization of the higher-order chromatin loop: specific attachment sites on nuclear scaffold. *Cell*, **39**, 223–232.
- Montoliu, L., Umland, T. and Schutz, G. (1995) A locus control region at –12 kb of the tyrosinase gene. *EMBO J.*, **15**, 6026–6034.
- Orkin, S.H. (1990) Globin gene regulation and switching: circa 1990. *Cell*, **63**, 665–672.
- Ortiz, B.D., Cado, D., Chen, V., Diaz, P.W. and Winoto, A. (1997) Adjacent DNA elements dominantly restrict the ubiquitous activity of a novel chromatin-opening region to specific region. *EMBO J.*, **16**, 5037–5045.
- Palmiter, R.D., Sandgren, E.P., Koeller, D.M. and Brinster, R.L. (1993) Distal regulatory elements from the mouse metallothionein locus stimulate gene expression in transgenic mice. *Mol. Cell. Biol.*, **13**, 5266–5275.
- Phi-Van, L., von Kries, J.P., Ostertag, W. and Stratling, W.H. (1990) The chicken lysozyme 5' matrix attachment region increases transcription from a heterologous promoter in heterologous cells and dampens position effects on the expression of transfected genes. *Mol. Cell. Biol.*, **10**, 2302–2307.
- Pikaart, M.J., Recillas-Targa, F. and Felsenfeld, G. (1998) Loss of transcriptional activity of a transgene is accompanied by DNA methylation and histone deacetylation and is prevented by insulators. *Genes Dev.*, **12**, 2852–2862.
- Poljak, L., Seum, C., Mattioni, T. and Laemmli, U.K. (1994) SARs stimulate but do not confer position-independent gene expression. *Nucleic Acids Res.*, **22**, 4386–4394.
- Pruzina, S., Hanscombe, O., Whyatt, D., Grosveld, F. and Philipsen, S. (1991) Hypersensitive site 4 of the human globin locus control region. *Nucleic Acids Res.*, **19**, 1413–1419.
- Robertson, G., Garrick, D., Wu, W., Kearns, M., Martin, D. and Whitelaw, E. (1995) Position-dependent variegation of globin transgene expression in mice. *Proc. Natl Acad. Sci. USA*, **92**, 5371–5375.
- Roseman, R.R., Pirotta, V. and Geyer, P.K. (1993) The su (Hw) protein insulates expression of the *Drosophila melanogaster* white gene from chromosomal position-effects. *EMBO J.*, **12**, 435–442.
- Scott, K.S. and Geyer, P.K. (1995) Effects of the su (Hw) insulator protein on the expression of the divergently transcribed *Drosophila* yolk protein genes. *EMBO J.*, **14**, 6258–6279.
- Smith, P.A. and Corces, V.G. (1995) The suppressor of Hairy-wing protein regulates the tissue-specific expression of the *Drosophila* gypsy retrotransposon. *Genetics*, **139**, 215–228.
- Spana, C., Harrison, D.A. and Corces, V.G. (1988) The *Drosophila melanogaster* suppressor of Hairy-wing protein binds to specific sequences of the gypsy retrotransposon. *Genes Dev.*, **2**, 1414–1423.
- Stramatoyannopoulos, J.A., Clegg, C.H. and Li, Q. (1997) Sheltering of γ -globin expression from position effects requires both an upstream locus control region and a regulatory element 3' to the Λ_γ -globin gene. *Mol. Cell. Biol.*, **17**, 240–247.
- Stief, A., Winter, D.M., Stratling, W.H. and Sippel, A.E. (1989) A nuclear DNA attachment element mediates elevated and position-independent gene activity. *Nature*, **341**, 343–345.
- Talbot, D., Descombes, P. and Schibler, U. (1994) The 5' flanking region of the rat *LAP* gene can direct high-level, position independent, copy number dependent expression in multiple tissues in transgenic mice. *Nucleic Acids Res.*, **22**, 756–766.
- Tuan, D., Solomon, W., Oiliang, L.S. and Irving, M.L. (1985) The 'β-like globin' gene domain in human erythroid cells. *Proc. Natl Acad. Sci. USA*, **82**, 6384–6388.
- Tuan, D., Abeliovich, A., Lee-Oldham, M. and Lee, D. (1987) Identification of regulatory elements of human β-like globin genes. In Stramatoyannopoulos, G. and Nienhuis, A.W. (eds), *Developmental Control of Globin Gene Expression*. Alan R. Liss Inc., New York, NY, pp. 211–220.
- Udvardy, A. and Schedl, P. (1993) The dynamics of chromatin condensation: redistribution of topoisomerase II in the 87A7 heat shock locus during induction and recovery. *Mol. Cell. Biol.*, **13**, 7522–7530.
- Udvardy, A., Maine, E. and Schedl, P. (1985) The 87A7 chromomere: identification of novel chromatin structures flanking the heat shock locus that may define the boundaries of higher order domains. *J. Mol. Biol.*, **185**, 341–358.
- Vasquez, J. and Schedl, P. (1994) Sequences required for enhancer blocking activity of scs are located within two nuclease-hypersensitive regions. *EMBO J.*, **13**, 5984–5993.
- Walters, M.C., Fiering, S., Eidemiller, J., Magis, W. and Groudine, M. (1995) Enhancers increase the probability but not the level of gene expression. *Proc. Natl Acad. Sci. USA*, **92**, 7125–7129.
- Walters, M.C., Magis, W., Fiering, S., Eidemiller, J., Scalzo, D., Groudine, M. and Martin, D.I. (1996) Transcriptional enhancers act *in cis* to suppress position-effect variegation. *Genes Dev.*, **10**, 185–195.
- Wijgerde, M., Grosveld, F. and Fraser, P. (1995) Transcription complex stability and chromatin dynamics *in vivo*. *Nature*, **377**, 209–213.
- Workman, J.L., Taylor, I.C.A. and Kingston, R.E. (1991) Activation domains of stably bound Gal4 derivatives alleviate repression of promoters by nucleosomes. *Cell*, **64**, 533–544.
- Zhao, K., Hart, C.M. and Laemmli, U.K. (1995) Visualization of chromosomal domains with boundary element-associated factor BEAF-32A. *Cell*, **81**, 879–889.

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