in obesity and establish *ADCY3* as a causal gene at the BMI-associated locus[2,4]. However, some important questions remain unanswered. Detailed phenotyping of homozygous *ADCY3* mutation carriers is needed to clarify whether additional multiorgan features, typical of ciliopathies, and the inability to smell are present. As *ADCY3* loss-of-function variants are rare in cosmopolitan populations, evaluating whether heterozygous carriers also develop a phenotype similar to that of haploinsufficient mice will be a challenging but worthy pursuit. In particular, the effect of carrying a burden of rare loss-of-function variants on type 2 diabetes risk will require validation in additional individuals. Nicely, both studies[3,5] demonstrate the value of studying diverse populations for genetic discovery and support expansion of such approaches.

Vaisse and colleagues[13] showed that MC4R and ADCY3 function and ciliary targeting in neuronal cells are critical for body weight regulation. Studying the effect of the newly described *ADCY3* variants[3,5] on ADCY3 targeting to primary cilia would be very interesting, as would investigating whether obesity-causal mutations in other ciliopathy-related genes affect MC4R or ADCY3 function and/or localization to neuronal cilia. Beyond its role in hypothalamic ciliated neurons, ADCY3 has a proposed broader peripheral function[7]; further studies investigating the impact of *ADCY3* mutations on its peripheral role in obesity (and type 2 diabetes) predisposition are obvious next steps. Lastly, ADCY3 has been proposed as an anti-obesity drug target[3,14]; however, *ADCY3* overexpression has also been linked to increased cell migration, proliferation and invasion in cancer[15], raising safety concerns. ❐

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Competing interests

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GENE REGULATION

Pioneering the chromatin landscape

New genomic analyses indicate that pioneer transcription factors can sample a diverse repertoire of common binding sites among different cell types and become enriched where they cooperate with other factors specific to each cell. Pioneer-factor binding is mechanistically separate from, and is necessary for, subsequent phenomena of chromatin opening and epigenetic memory in vivo.

Kenneth S. Zaret

The pioneer-factor concept emerged from in vivo footprinting studies seeking to determine which transcription factors are the first to bind a tissue-specific enhancer during embryonic development. Among the footprint sites assessed in undifferentiated endoderm germ-layer cells, nascent hepatoblast progenitors and adult hepatocytes, binding sites for FOXA and GATA transcription factors are the only ones occupied before hepatic induction, when the target gene *Alb*, encoding albumin, is silent[1,2]. FOXA1 and FOXA2 are necessary for endoderm differentiation into hepatoblasts[3] and thus act as competence factors[1]. Subsequent transcription factors crucial for *Alb* enhancer activity are recruited in hepatoblasts as *Alb* turns on[1]. Recombinant FOXA1 protein and, to a lesser extent, GATA4 have an intrinsic biochemical ability to bind their target sites on nucleosomes in vitro, whereas subsequent binding transcription factors do not[4]. Finally, recombinant FOXA1 and GATA4 are sufficient to expose target DNA in a compacted nucleosome array in the absence of an ATP-dependent nucleosome remodeler. These findings have led to the proposal that FOXA1 and GATA4 are pioneer transcription factors that target nucleosomal DNA and program naive chromatin[5]. However, several questions remained: How do these pioneer factors scan the genome and become enriched at target sites? Is their initial binding in vivo accompanied by chromatin opening, and what are the epigenetic consequences of pioneer-factor binding? These questions and others have been addressed in two new studies by Meissner and colleagues[6] and Drouin and colleagues[7].

Sampling sites in chromatin

If pioneer factors can dominantly engage silent target sites in chromatin, why have genome-site-mapping studies shown that FOXA factors occupy only a subset of the same sites in different breast cancer cell lines[8]? Concordantly, Donaghey et al.[6] have found that, in HepG2 hepatoma cells, A549 lung carcinoma cells and endoderm derived from human embryonic stem cells (ESCs), endogenous FOXA2 engages many sites in common but also engages many sites differentially. Further differences were observed when FOXA2 was ectopically expressed in human fibroblasts[6]. Ectopic GATA4 and the pluripotency factor OCT4 in fibroblasts also bound only a subset of sites in common with those bound by endogenous factors in ESC-derived endoderm and ESCs, respectively[6]. However, after curation of the genomic data to decrease the significance threshold for mapping binding events, FOXA2 and GATA4 expressed ectopically in human fibroblasts and FOXA2 in

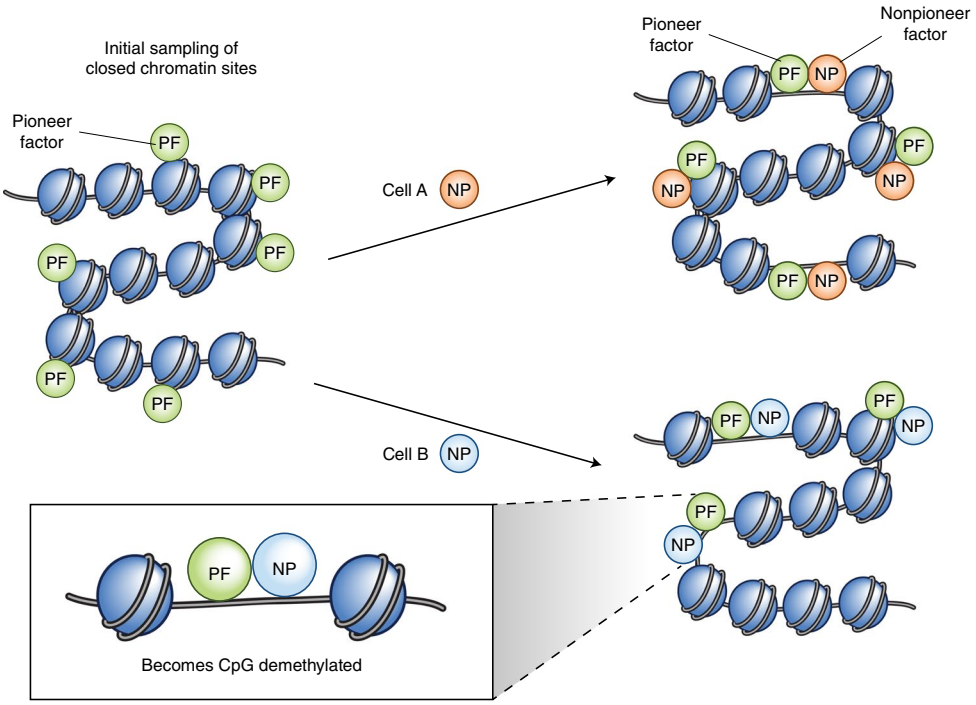

Fig. 1 | Dynamics of pioneer-factor activity. Pioneer factors such as FOXA2 can sample many genomic sites in closed chromatin. At a subset of such sites, pioneer factors exhibit stronger binding through cooperative interactions with cell-specific nonpioneer factors, thus leading to demethylation of CpG dinucleotides in the underlying DNA.

endogenous HepG2 and ESCs weakly occupied most target sites that exhibited strong engagement in the other cell lines tested. In other words, in each cell line, FOXA2 and GATA4 sample diverse sites in common across the different cell genomes, and they are further enriched at cell-specific sites depending on interactions with transcription factors particular to each cell type. Whereas other studies have shown that 5% of FOXA binding events at nonmotif sites can be initiated with assisted loading by other factors[9], the new findings suggest that FOXA2 and GATA4 factors indeed sample most target sequences independently of other factors and that cooperative interactions drive more stable occupancy (Fig. 1).

How do pioneer factors sample sites in the genome? Fluorescence recovery after photobleaching (FRAP) has shown that FOXA1, like all other transcription factors studied to date[10], is mobile in the nucleus[11]. However, FOXA1 exhibits slower FRAP than do many other factors, thus indicating a lower nuclear mobility that correlates with higher nucleosome binding in vitro[11]. Given that most nuclear DNA is nucleosomal, the low nuclear mobility of pioneer factors seems to be due to lateral diffusion sampling of the abundant nucleosomal sites in chromatin, whereas the high nuclear mobility of nonpioneer factors seems to be due to far fewer interaction sites and thus higher nucleoplasm diffusion[11]. The analysis by Donaghey et al.[6] links these findings by indicating that pioneer-factor scanning of chromatin includes strong binding events at cell-specific sites, weaker sampling events common to many cell types, nonspecific nucleosome scanning events, as seen by FRAP, and open-site interactions. The complexity of binding events illustrates the difficulty of interpreting residence-time values from single-molecule tracking studies in vivo[9].

Who's on first?

Despite the extensive genomic studies on pioneer factors[12], it has been unclear whether nucleosome remodelers, chromatin modifications or pioneer factors act first in vivo. Mayran et al.[7] have analyzed pituitary melanotrope cells, whose specification is dependent on pioneering by the transcription factor Pax7, by using an estrogen receptor (ER)-Pax7 fusion protein whose activity can be induced acutely by hormone. Within only 30 minutes of induction in corticotrope cells, ER-Pax7 was detected at active and inactive ('pioneered') enhancer targets, the latter of which were assay for transposon-accessible chromatin (ATAC–seq) negative and lacked the histone modifications typical of active enhancer chromatin. At active enhancers, binding was as high at 30 minutes as it was at 3 days, and nearby genes exhibited rapid transcriptional responses. At pioneered targets, binding was evident at 30–60 minutes and became stronger at 3 days. Yet, on the basis of ATAC–seq, the chromatin remained closed after initial pioneer-factor binding but became increasingly accessible over 3 days, and RNA induction of nearby genes followed suit. Mayran et al.[7] conclude that Pax7 binds rapidly to its pioneer-targeted sites and that chromatin opening in vivo, along with gene activation, is a slower secondary process in this developmentally regulated system.

Mayran et al.[7] have further shown that ER-Pax7 binding leads to loss of CpG methylation at pioneered enhancers in pituitary corticotrope cells, and subsequent loss of Pax7 does not allow enhancer CpG methylation to recover over 12 cell divisions. Thus, initial pioneer binding in this system leads to epigenetic persistence of CpG methylation changes (Fig. 1). Donaghey et al.[6] have also observed loss of CpG methylation after FOXA2 induction in fibroblasts. Preferential loss was seen proximal to or within a FOXA2 binding event, and FOXA2 binding in S phase appeared to be necessary for methylation loss, thus suggesting a passive mechanism whereby FOXA2 binding may prevent

maintenance methylation and thereby elicit an epigenetic change.

Mayran et al.[7] have also observed Pax7 pioneer binding to sites of unmarked chromatin that did not become opened or activated. Are these unproductive events, or do they reflect competence for other genetic responses? Oct4 and Sox2 are both pluripotency conversion factors[13] and pioneers, but how does only Sox2 exhibit a marked increase in sampled sites when genomic binding data are viewed at lower thresholds[14]? How does chromatin opening by pioneer-factor binding to compacted chromatin in vitro relate to enabling secondary events in a living cell? Clearly, there is much more to know! ❐

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Competing interests

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CANCER METABOLISM

Genetics of lipid metabolism in prostate cancer

Dysregulated lipid metabolism is a prominent feature of prostate cancers. Two papers in this issue identify novel genomic drivers of lipid metabolism in prostate cancer and provide implications for the subtyping and treatment of the disease.

Ninu Poulose, Francesca Amoroso, Rebecca E. Steele, Reema Singh, Chee Wee Ong and Ian G. Mills

Prostate cancer is a high-incidence cancer characterized in part by dysregulated lipid metabolism. This dysregulation is a prominent feature that encompasses elevated de novo lipogenesis including steroid-hormone biosynthesis as well as beta-oxidation of fatty acids[1]. In imaging prostate cancer, the importance of lipid metabolism is evidenced by the adoption of choline- and acetate-based tracers for the detection of alterations in situ[2]. Furthermore, fatty acid synthase has previously been reported to have the properties of a prostate cancer oncogene, as assessed in a transgenic mouse model of prostate cancer[3]. In addition, inhibitors of fatty acid synthase have been found to restrict prostate cancer growth, at least in preclinical cancer models, and these findings have prompted considerable interest in drug repurposing and further development of these therapeutics[4,5]. Epidemiological data have implicated obesity as a risk factor for aggressive prostate cancer[6]. Collectively, these findings imply that lipid metabolism is a major contributor to sustaining prostate cancer development, and there is a major need for further mechanistic insights into the drivers of lipid metabolism, be they genetic or environmental.

In this issue, two studies by Alimonti and colleagues[7] and Pandolfi and colleagues[8] identify novel regulatory effects of genomic changes in prostate cancer on the ability of prostate cancer cells to metabolize lipids (Fig. 1). Both studies use the same prostate-specific *Pten*-null transgenic mouse model of prostate cancer, in which mice develop high-grade intraepithelial prostate tumors at an early age and invasive prostate cancer at a late age[7,8].

The study by Alimonti and colleagues[7] focuses on the frequent amplification and overexpression of subunits of the pyruvate dehydrogenase complex (PDC), which has a gatekeeper function in converting pyruvate into acetyl CoA for entry into the tricarboxylic acid cycle in mitochondria[7]. Having established that overexpression of subunits of the PDC is a feature of both clinical prostate cancer and the *Pten*-null transgenic mouse model, the authors showed that inactivation of *Pdha1* restrains prostate growth at early ages in this model[7]. The underlying mechanistic basis involves both the nuclear and mitochondrial functions of this complex[7]. Previous studies have reported that acetyl CoA is not required merely to sustain the metabolic activity of mitochondria but is additionally required in the nucleus to support histone acetylation and enhancer activity[9]. By using a combination of metabolomic and transcriptomic profiling, the authors show that a principal effect of targeting the PDC complex, particularly PDHA1, is suppression of lipid biosynthesis. This effect was found to be due at the nuclear level to decreased histone acetylation at regulatory regions bound by the SREBP transcription factor, and at the mitochondrial level to decreased citrate production[7]. This study identifies the PDC complex, particularly PDHA1, as a potential therapeutic target through which prostate cancer development can be restrained through direct and indirect effects on the metabolic capacity of prostate cancer cells[7].

In the other study, Pandolfi and colleagues[8] set out to explore the effects of co-deletion of the tumor suppressors Pml and Pten on the phenotype of *Pten*-null prostate cancers, identifying lipid metabolism as the amplified biological process arising from coordinate targeting of both Pml and Pten[8]. They determined that this metabolic change reflects hyperactivation of an SREBP-dependent prometastatic lipogenic program[8] reminiscent of the nuclear/transcriptional changes observed by Alimonti and colleagues[7]. Enhanced transcription of SREBP-target genes occurs through the hyperactivation of mitogen-activated protein kinase (MAPK) signaling, which is otherwise restrained by Pml[8]. The metastatic